Scientific Abstract

In 1993, a State-Of-The-Art conference was convened by the NIAID to evaluate the role of nucleoside analog reverse transcriptase inhibitors (AZT, ddI, ddC) in the treatment of HIV-1 infection. The finding of the panel underscored the emerging concerns of the limited effectiveness and durability of response to these drugs. At route of the limited effectiveness of the RT inhibitors may be the inherent difficulty of treating "retroviral infections" since the integrated provirus becomes an "inheritable" feature of the cell and retains the potential for viral expression and production of progeny virus for the duration of the cell's lifetime. In essence, HIV infection results in an acquired genetic disease which may be best treated by a genetic based strategy.

Human immunodeficiency virus (HIV) productively infects human $CD4^+$ T cells and causes their progressive depletion. Depletion of $CD4^+$ cells results from the combined direct cytopathic effects of HIV-1 and from inappropriate auto-immune destruction of uninfected $CD4^+$ cells. Both destructive processes appear to be the result, at least in part, of cellular exposure to the HIV-1 envelope glycoprotein, gp120.

We have recently reported that intracellular antibodies, termed intrabodies, may be useful for the gene therapy of HIV-1 infection and have demonstrated the feasibility of using intrabodies to inhibit the function of the HIV-1 envelope glycoprotein. The broadly neutralizing human monoclonal antibody F105 that competes with CD4 for binding to gp120 was engineered to create an intrabody (sFv105) that would be retained in the lumen of the endoplasmic reticulum (ER) of the $CD4^+$ T cell where it would bind to the nascent folded envelope protein within the ER and prevent transit of the envelope antibody complex to the cell surface. These studies showed that sFv105 was stably expressed and retained in the ER and was not toxic to the cells. Cleavage and maturation of gp160 (which is required to produced infectious HIV-1 virions) was inhibited, markedly diminished syncytium formation was observed and the infectivity of the HIV-1 particles produced by HIV-1-infected cells was substantially reduced (1,000 to 10,000-fold). Down-regulation of the surface CD4 by HIV-1 was not observed in the lymphocytes intracellularly expressing sFv105. Cell surface phenotype, replication rate, morphology, and response to mitogenic stimulation of the transformed cells were also normal. Moreover, the F105 intrabodies were also able to inhibit infectious HIV-1 production by binding intracellularly to envelope mutant viruses that escape neutralization by extracellular antibody.

The principal objectives of this feasibility study of somatic cell gene transfer into healthy, asymptomatic HIV-1-infected humans are to evaluate the safety of infusing autologous lymphocytes that have been transduced *ex vivo* with a retroviral vector encoding the human sFv105 intrabody against HIV-1 envelope protein. We will compare *in vivo* in each patient the kinetics and survival of sFv105-transduced cells with a separate aliquot of cells transduced with a control vector (identical except for the sFv105 cassette). We will evaluate the *in vivo* expression of sFv105 in transduced lymphocytes. We will also investigate whether host immune responses directed against the transduced cells will occur *in vivo*. Finally, we will make preliminary observations on the effects of gene therapy with the sFv105 single chain antibody on *in vivo* viral mRNA expression, viral burden, and $CD4^+$ lymphocyte levels. The results will determine whether this intracellular antibody can protect $CD4^+$ T cells in patients with HIV-1 infection.

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